

The slide to be stained for CD3 comprised:

1: Liver, 2: Tonsil fixed 4 h, 3: Tonsil fixed 48 h, 4: Tonsil fixed 96 h, 5-7: Nodal T-cell lymphomas.

Criteria for assessing a CD3 staining as optimal included:

- A strong and distinct predominantly membranous reaction of the majority of neoplastic cells in the 3 T-cell lymphomas
- A strong and distinct predominantly membranous staining of all normal T-cells in the tonsils and liver
- No staining in other cells. Especially B-cells in the tonsil should be negative



87 laboratories participated in the assessment. 29 achieved optimal staining (33 %), 35 good (40 %), 20 borderline (23 %) and 3 (5 %) poor staining.

The following Abs were used:

mAb clone **F7.2.38** (DakoCytomation n=33)

mAb clone **PS1** (Novocastra n=20, Ventana n=12)

mAb clone **SP7** (NeoMarkers n=7)

mAb clone **UCHL1** (DakoCytomation n=1)

pAb **A0452** (DakoCytomation n=11)

pAb **NCL-CD3p** (Novocastra n=2)

pAb **RB-360** (NeoMarkers n=1)

Optimal staining in this assessment was obtained with following Abs: clone **F7.2.38** (11 out of 33 were optimal), clone **PS1** (10 out of 32 were optimal), clone (rabbit) **SP7** (2 out of 7 were optimal), pAb **A0452** (5 out of 11 were optimal), and pAb **RB-360** (1 out of 1 was optimal).

All optimal protocols were based on HIER irrespectively of the Ab employed.

With clone **F7.2.38** only Tris-EDTA/EGTA pH 9 was used as HIER buffer in optimal protocols. F7.2.38 diluted 1:50 – 1:100 combined with HIER in Tris-EDTA/EGTA pH 9 resulted in an optimal staining in 11 out of 23 laboratories.

With clone **PS1** the following HIER buffers were used in optimal protocols: Tris-EDTA/EGTA pH 9 (8 out of 16 were optimal), EDTA pH 8 (1 out of 3 was optimal) and Citrate pH 6 (1 out of 2 was optimal). This Ab could also be used as a Ready-To-Use product. Using a concentrated Ab diluted 1:50-100, 8 out of 18 obtained an optimal staining, while only 2 out of 12 using a Ready-To-Use Ab obtained an optimal staining.

With clone **SP7** only Tris-EDTA/EGTA pH 9 could be used as HIER buffer (2 out of 3 were optimal). For an optimal result, SP7 was either diluted 1:100 using a water bath or 1:800 using a pressure cooker as heating device for HIER.

With pAb **A0452** the following buffers were used for an optimal result: Tris-EDTA/EGTA pH 9 (4 out of 9 were optimal) and EDTA/EFTA pH 8 (1 out of 2 was optimal). The pAb was typically diluted in the range of 1:300 – 1:400.

pAb **RB-360** was used with HIER in Tris-EDTA/EGTA pH 9 and diluted 1:200 giving an optimal result.

The most frequent causes of insufficient stains were (often in combination):

- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- False positive reaction due to endogenous biotin

- Insufficient as well excessive epitope retrieval
- Apparently inappropriate choice of primary Ab

The prevalent feature of an insufficient staining was a too weak staining or false negative staining of the T-cells. Also a false positive reaction due to endogenous biotin was observed as a common feature, primarily in liver cells

and appendiceal columnar cells. This was typically seen in staining combining efficient HIER in an alkaline buffer as Tris-EDTA/EGTA pH 9 or EDTA pH 8 and a biotin based detection system.

The T-cells in the tonsils should stain as intensely as possible without reaction of the B-cells. A good quality indicator was the germinal centres, in which the isolated T-cells should be distinctively labelled with no reaction of the B-cells. In general using a biotin based detection system without biotin blocking, HIER in Citrate pH 6 is preferable to Tris-EDTA/EGTA pH 9 to minimize the appearance of endogenous biotin. A higher concentration of the primary Ab should be used to compensate for the lower sensitivity.

CD3 was also assessed in run 5. In that run 45 laboratories participated, out of which 14 (31 %) were assessed as insufficient, close to the present proportion (28%). However, many laboratories participated in the CD3 assessment for the first time.

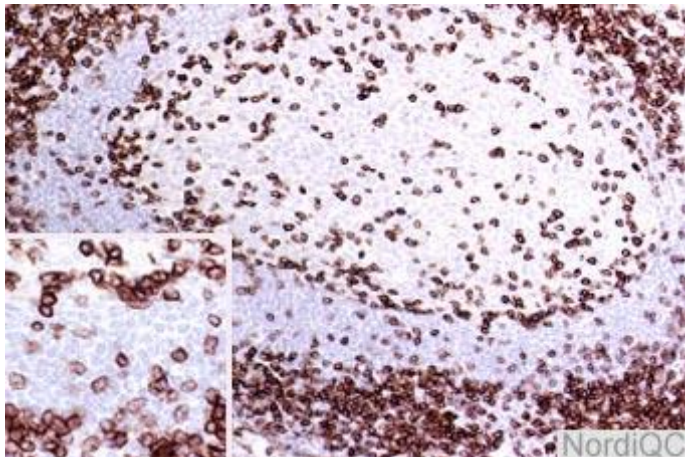


Fig. 1a  
Optimal staining for CD3 in a normal tonsil. The T-cells show a strong membranous staining. Both grouped T-cells and - more importantly - isolated T-cells in the B-zone are distinctively demonstrated (see inset).

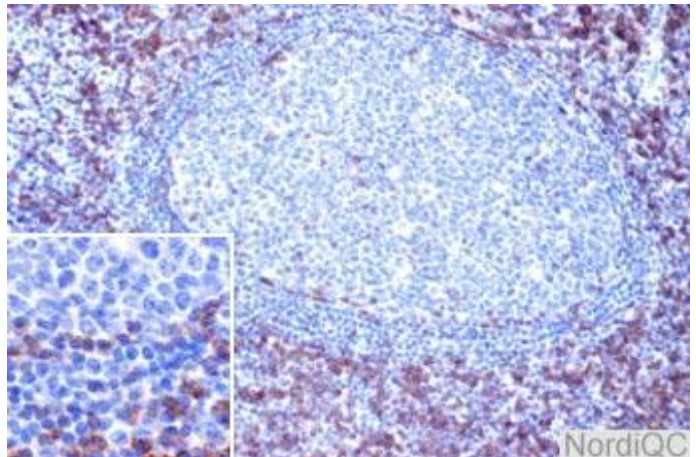


Fig. 1b  
Insufficient staining for CD3 in a normal tonsil (same field as in Fig. 1a). The T-cells only show a diffuse and weak staining. The isolated T-cells in the B-zone are almost negative (see inset).

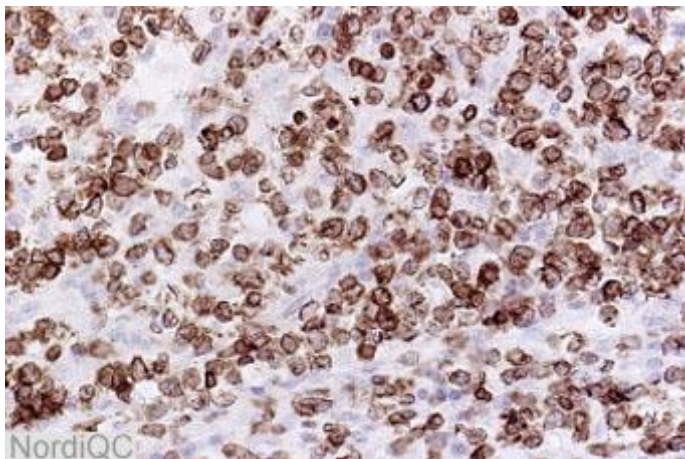


Fig. 2a  
Optimal staining for CD3 in a T-cell lymphoma. Almost all the neoplastic cells show a moderate to strong and distinct staining.

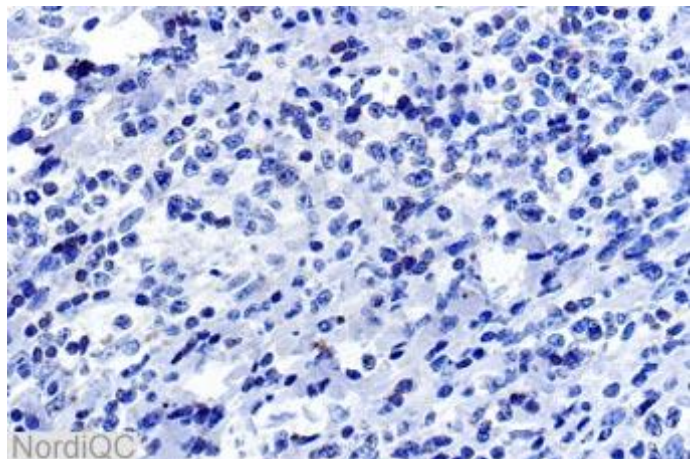


Fig. 2b  
Insufficient staining for CD3 in a T-cell lymphoma (same field as in Fig. 2a). The neoplastic cells are virtually negative, while only few residual normal T-cells are stained.

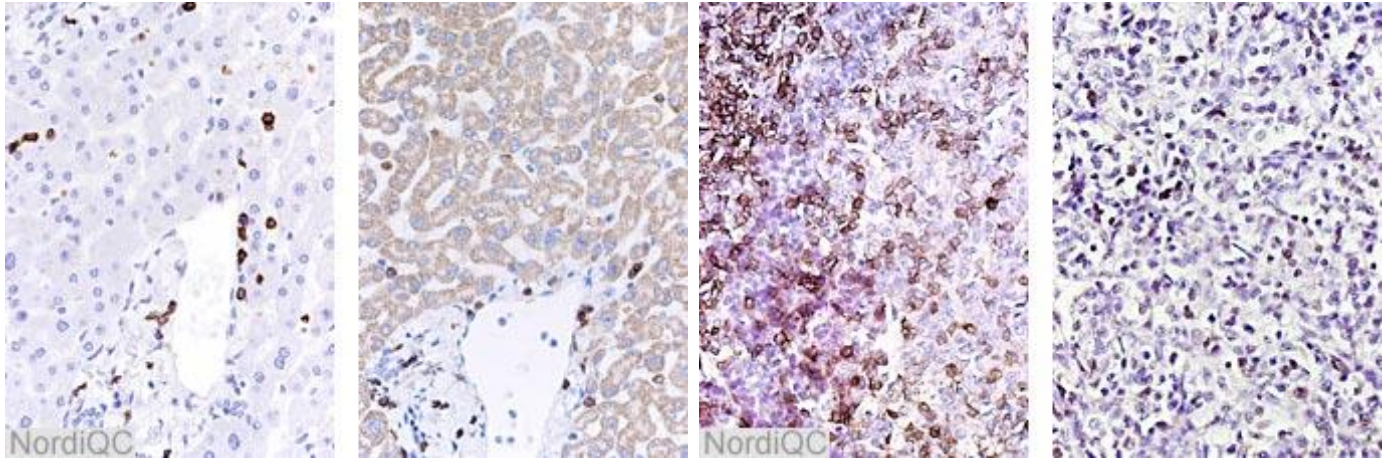


Fig. 3a  
 Left. Optimal staining for CD3 in the liver. Only the T-cells are demonstrated without any reaction in other cells.  
 Right. Insufficient staining for CD3 in the liver. The T-cells are demonstrated, but all of the liver cells are stained due to a false positive reaction of endogenous biotin (LSAB combined with HIER in an alkaline buffer).

Fig. 3b  
 Insufficient staining for CD3 in a normal tonsil (left) and in a T-cell lymphoma (right). The reaction is very diffuse and the morphology is impaired (compare to fig. 1a and 2a) due to excessive HIER.

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